REMARKS

I. Status of the Application

Claims 10 and 13-28 are pending in the application. Applicants gratefully acknowledge the Examiner's withdrawal of her objection to the drawings, and her rejections of claim 10 under 35 § U.S.C. 112, second paragraph, and under 35 § U.S.C. 102(b) as anticipated by Cantor et al. Claims 10 and 13-19 stand rejected under 35 § U.S.C. 112, first paragraph. Claims 20-28 stand rejected under 35 § U.S.C. 102(b) as anticipated by Gronthos et al., (1994) Blood 84:4164.

Applicants have amended the claims to more clearly define and distinctly characterize Applicants' novel invention. Support for the amendments can be found in the specification and the claims as originally filed. Support for the amendments to claims 20 and 25 to recite "culture medium" can be found in the specification at least at page 4, line 10. Support for the amendments to claims 20 and 25 to recite "removing the substrate and the cells from the culture medium," can be found in the specification at least at page 4, lines 8 and 9 (for removing the substrate from the culture medium), and at page 3, lines 26-29 (for medium where cells have previously been incubated). Claims 14, 23 and 28 were amended to remove the language "bone remodeling factors."

Applicants respectfully request entry and consideration of the foregoing amendments, which are intended to place this case in condition for allowance.

II. The Specification Provides Adequate Written Description for Claims 10 and 13-19

At page 3, paragraph 2 of the instant Office Action, claims 10 and 13-19 stand rejected under 35 U.S.C. § 112, first paragraph, as not containing a written description of the claimed

invention. The Examiner is of the opinion that the specification does not provide support for "A method of producing active factors comprising the steps of: (b) contacting the cells with a culture medium for a sufficient time to produce a matrix." The Examiner asserts that the instant claims now recite limitations which were not clearly disclosed in the specification as filed, and now change the scope of the instant disclosure.

Applicants traverse this rejection. Applicants respectfully submit that the specification provides adequate support for the limitations of claim 10 and claims depending therefrom. The Examiner's attention is respectfully directed to Applicants' working example 3, where Applicants set forth a method of producing "osteoblast derived factors" (page 6, line 32) (*i.e.*, ***Applicants teach a method of producing active factors***). At page 8, lines 4-6, Applicants teach that their method produced "factors produced by osteoblasts that have a stimulating effect on osteoclastic activity." At page 7, lines 6-12, Applicants teach a method of producing these factors in osteoblast cultures by incubating rat bone marrow cells in α-minimum essential medium supplemented with fetal bovine serum, antibiotics, ascorbic acid, dexamethasone, and β-glycerophosphate for 18 days. At page 5 of the specification, Applicants teach that the growth of bone marrow cells in the above-described medium (lines 2-6) produces nodular structures comprising matrix (lines 23-24) from approximately ***2 weeks*** onward (lines 14-16). Thus, incubating the cells in this medium for ***18 days***, as set forth in example 3, is a sufficient time period to produce matrix (*i.e.*, ***Applicants teach contacting the cells with a culture medium for a sufficient time to produce a matrix***).

Thus, Applicants' specification provides support for "A method of producing active factors comprising the steps of: (b) contacting the cells with a culture medium for a sufficient

time to produce a matrix." Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

III. Claims 20-28 Are Novel Over Gronthos et al.

At page 4, paragraph 2 of the instant Office Action, claims 20-28 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Gronthos et al., (1994) Blood 84:4164. The Examiner is of the opinion that Gronthos et al. teaches the culturing of bone marrow stromal cell progenitors from adult human bone marrow and that these cells were capable of forming a layer *in vitro* consisting of stromal cell types. The Examiner asserts that the cells expressed alkaline phosphatase, which is a well documented marker of bone cell differentiation, and that adherent layers of bone marrow cultures displayed large areas of mineralized material. The Examiner further asserts that samples were taken from the medium of cultures and analyzed for the presence of osteocalcin. Applicants respectfully traverse this rejection in view of the amended claims now presented.

Amended claim 20 and claims depending therefrom are directed to a method of producing bone growth factors comprising the steps of applying bone marrow cells on a substrate, contacting the bone marrow cells with a culture medium for a sufficient time to produce bone growth factors, removing the substrate and the cells from the culture medium, and ***recovering the bone growth factors from the culture medium***. Amended claim 25 and claims depending therefrom are directed to a method of producing growth factors comprising the steps of applying stromal cells on a substrate, contacting the stromal cells with a culture medium for a sufficient time to produce growth factors, removing the substrate and the cells from the culture medium, and ***recovering the growth factors*** from the culture medium.

Applicants' claimed method is directed to producing and recovering growth factors in the context of cultured bone marrow cells and stromal cells. Applicants teach that the active factors recovered are useful, for example, to enhance the functioning and adaptation of surgical implants (page 4, lines 16-19).

Gronthos et al. is directed to establishing an *in vitro* culture system to demonstrate the osteogenic potential of fibroblast colony-forming units derived from human bone marrow (BM CFU-F cells) (page 4165, left column, first full paragraph). Gronthos et al. states that their cultured cells "exhibit three independent markers of differentiated cells: alkaline phosphatase expression;...the bone specific protein, osteocalcin; and production of a mineralized matrix (hydroxyapatite)" (page 4165, left column, first full paragraph, emphasis added).

Regarding alkaline phosphatase detection, Gronthos et al. teaches immunoperoxidase ***staining of*** BM CFU-F ***cells*** (page 4167, bottom of left column, top of right column). The protocol of Gronthos et al. entails binding monoclonal antibody to cells, ***washing away the culture media***, and fixing the cells in paraformaldehyde for microscopy (page 4165, right column, last full paragraph). The alkaline phosphatase was thus identified ***on fixed cells*** (i.e., on tissue culture dishes or slides). Gronthos et al. does not teach or suggest removing the substrate and the cells from the culture medium and then recovering alkaline phosphatase from the culture medium.

Gronthos et al. also teaches assaying the presence of mineralized materials by Von Kossa staining using fixed, imbedded cells (page 4167, right column, last paragraph; page 4165, right column, last paragraph). Gronthos et al. teaches teasing cell layers off of the substrate, fixing the cell layers in ethanol, and embedding the cells layers in glycol methacrylate/methyl methacrylate. Finally, Gronthos et al. teaches the visualization of mineralized materials ***in the***

fixed/embedded cells using light microscopy. Gronthos does not teach or suggest removing the substrate and the cells from the culture medium and then recovering mineralized materials from the culture medium.

The Examiner asserts that Gronthos et al. teaches taking samples of culture medium and analyzing them for the presence of osteocalcin. Applicants submit that Gronthos et al. neither teaches nor suggests that osteocalcin is a growth factor, and is not concerned with recovering it as such. Instead, Gronthos et al. assays is interested in assaying the presence of osteocalcin merely "as a ***marker*** of differentiated bone cells" in order to show that their cultured cells can form osteoblasts (page 4165, left column, first full paragraph, emphasis added). Furthermore, osteocalcin is not widely accepted in the art as a growth factor. In fact, there is evidence in the art that osteocalcin actually ***inhibits bone formation*** (see Attachment A, Bronckers et al., 1998, Eur. J. Oral Sci.). Thus, Gronthos et al. fails to teach or suggest recovering growth factors from the culture medium.

Even assuming, arguendo, that osteocalcin is a growth factor, unlike the growth factors recovered by Applicants' methods, the methods of Gronthos et al. do not provide osteocalcin in a form which would be suitable for later use in cell culture applications. The only teaching by Gronthos et al. of analyzing the osteocalcin protein is in the context of a radioimmunoassy (RIA) (page 4166 right column, first full paragraph). Gronthos et al. teaches that RIA requires a binding of osteocalcin to an antibody. Antibody binding would likely alter the ability of osteocalcin to interact with other proteins. For instance, the antibody could bind to an active site of osteocalcin, it could change the conformation of osteocalcin, or it could prevent osteocalcin from undergoing conformational changes necessary for mediating potential cellular functions. Furthermore, during the RIA procedure osteocalcin would be incubated in RIA buffer which

typically contains sodium azide and/or detergents. Sodium azide is highly toxic and would thus render the samples unsuitable for downstream *in vivo* use. Detergents would serve to alter the native conformation of the osteocalcin protein, and thus could effect its activities. Finally, the RIA procedure would make the osteocalcin radioactive, further rendering the protein unsuitable for *in vivo* use.

For at least these reasons, Applicants submit that Gronthos et al. fails to teach each and every element of Applicants' claimed subject matter. Accordingly, Applicants respectfully request that the rejection of claims 20-28 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

IV. Conclusion

Having responded to all outstanding issues, reconsideration and allowance of all the pending claims is respectfully requested. If a telephone conversation with Applicants' attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 720-9600.

Respectfully submitted,

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